A safe, blood-brain barrier permeable triphenylmethane dye inhibits amyloid-beta neurotoxicity by generating non-toxic aggregates

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Supporting Information (SI)

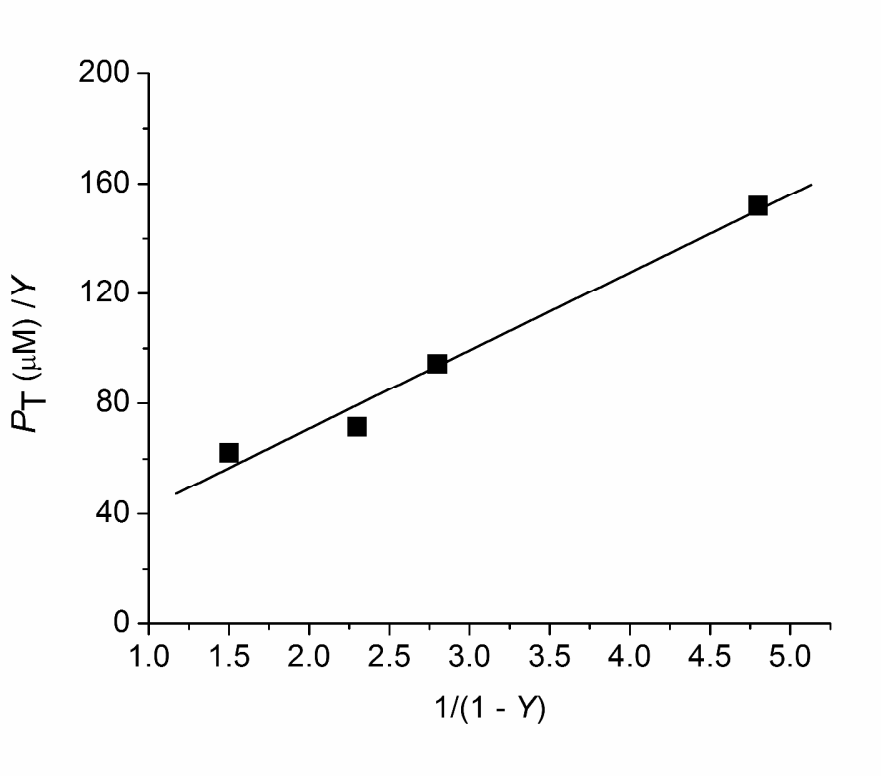

Figure S1.

Figure S1. The data on the BBG-Aβ binding saturation curve (Figure 7) were fitted into a straight-line according to the equation, $P_T/Y = 1/(nk)\ [1/(1 - Y)] + D_T/n$ derived from $Y = nk(D)(P_T/D_T)/(1 + k(D))$ where Y, n, k, D, D_T, and P_T mean the fractional saturation of ligand binding sites, the number of binding sites, the binding constant, the dye concentration in solution, the total dye concentration, and the total protein concentration, respectively (*1*). The BBG concentration (D_T) was fixed at 49 μM. The Aβ concentration (P_T) was varied from 0 to 300 μM. The data at four P_T values between 20 μM and 120 μM were used for the fitting to a straight-line ($R^2 = 0.98$). The values of n and k were 3.2 and $1.1*10^4$ (M^{-1}), respectively.

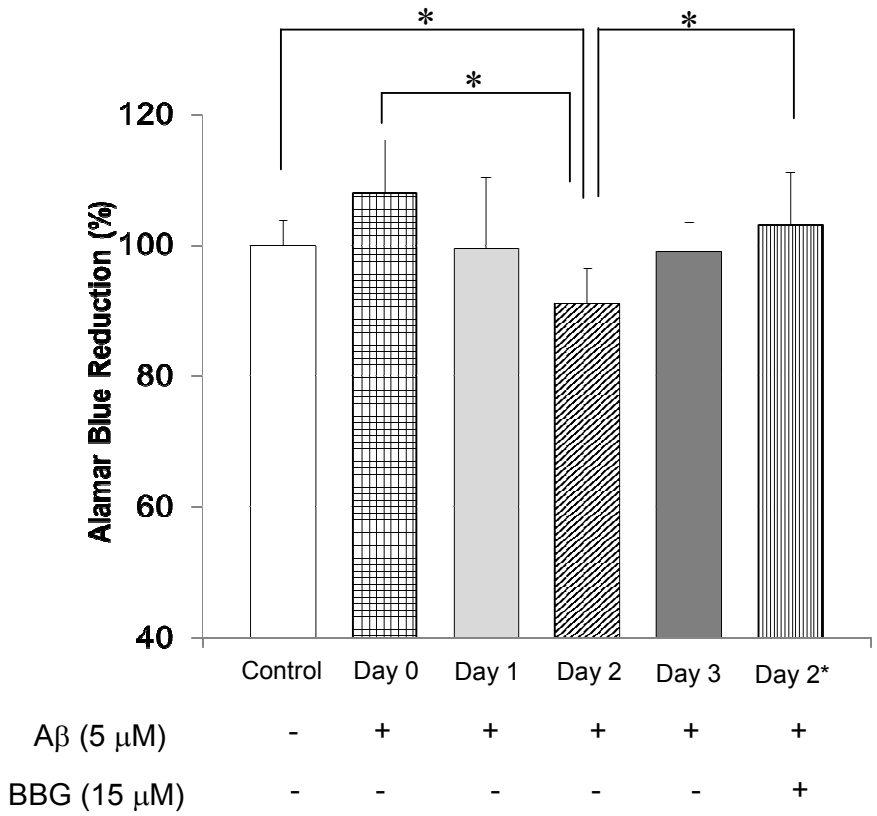

Figure S2

Figure S2. Alamar blue reducing activities of neuroblastoma SH-SY5Y cells incubated with pre-formed Aβ samples in the absence or presence of 3x BBG. Preformed Aβ aggregates were prepared by incubating 50 μM of Aβ monomer in the absence of BBG at 37 °C for 0 to 3 days or in the presence of 3x BBG for 2 days, as indicated in the graph. The Aβ aggregates were then administered to SH-SY5Y cells at a final concentration of 5 μM. After 3 days, cell viability was measured using alamar blue reduction. Cells administered with PBS as a control (*Control*), Aβ incubated without BBG for 0 (*Day 0*), 1 (*Day 1*), 2 (*Day 2*), or 3 days (*Day 3*), or Aβ incubated with 3x BBG for 2 days (*Day 2**). Values represent means ± standard deviation ($n \geq 3$). Values are normalized to the viability of cells administered with PBS only. One-sided Student's t-tests were applied to the data. * $P < 0.05$.

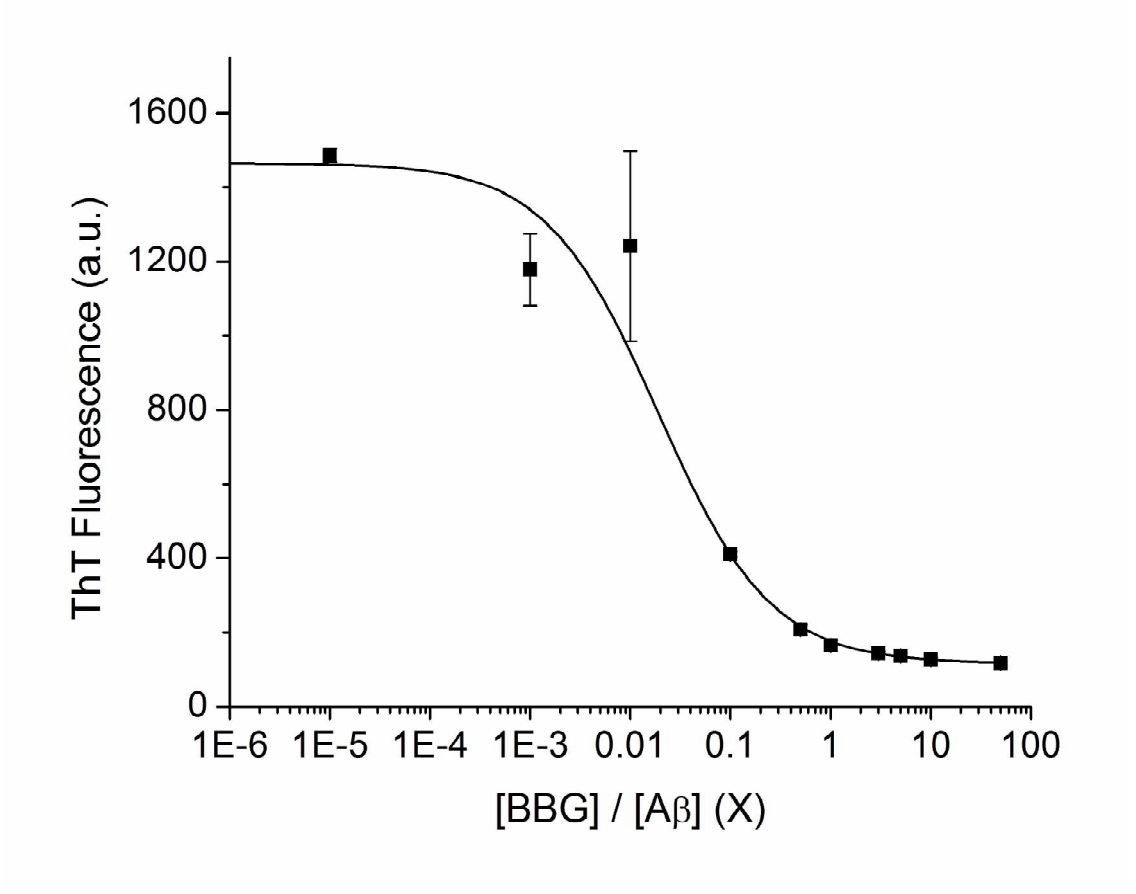

Figure S3.

Figure S3. Dose-dependence inhibition of ThT fluorescence of Aβ samples by BBG. 50μM of Aβ monomer was incubated at 37 °C in the absence (no BBG) or presence of the indicated concentrations of BBG (from 10^{-5}x to 50x). 5 μL of Aβ sample was taken at 72 hours of incubation. ThT fluorescence was measured in arbitrary units (a.u.). Values represent means ± standard deviation (n = 3). The data were fitted to a sigmoid curve (R^2 = 0.99).

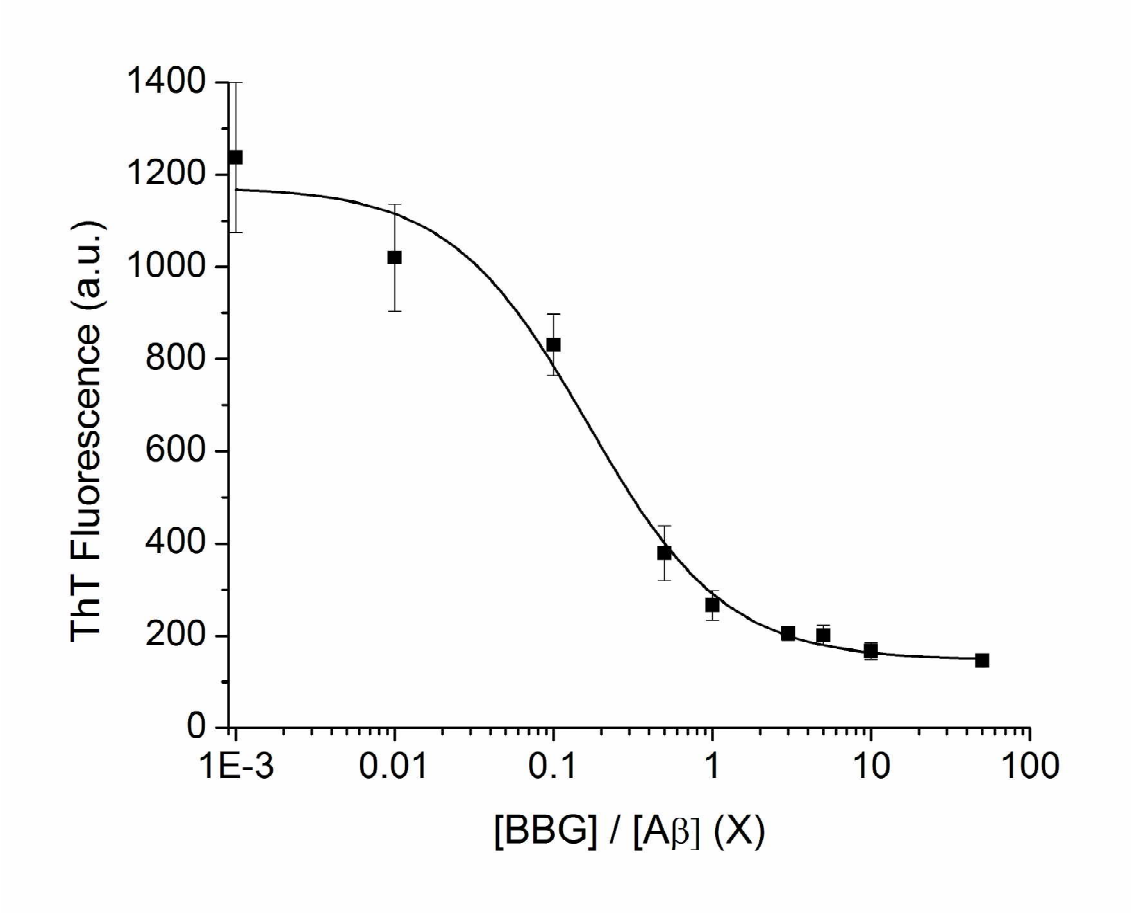

Figure S4.

Figure S4. ThT fluorescence of preformed amyloid fibrils (72 hrs) mixed with varying concentrations of BBG immediately prior to adding ThT. ThT fluorescence was measured in arbitrary units (a.u.). Values represent means ± standard deviation (n = 3). The data were fitted to a sigmoid curve ($R^2 = 0.99$).

References

1. Sohl, J. L., and Splittgerber, A. G. (1991) The binding of Coomassie brilliant blue to Bovine Serum Albumin: A physical biochemistry experiment, *J. Chem. Educ. 68*, 262-null.